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TRANSPLANTATION AMELIORATES THE HYPERCOAGULABILITY OF END-STAGE RENAL DISEASE

Fred Aslan

YALE UNIVERSITY

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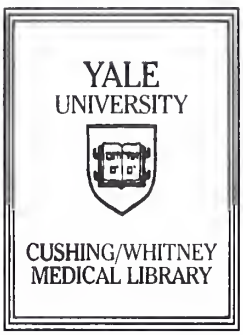
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TRANSPLANTATION AMELIORATES THE HYPERCOAGULABILITY OF ENDSTAGE RENAL DISEASE

A Thesis Submitted to the

Yale University School of Medicine

in Partial Fulfillment of the Requirements for the

Degree of Doctor of Medicine

By

Fred Aslan

2002

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ABSTRACT

TRANSPLANTATION AMELIORATES THE HYPERCOAGULABILITY OF ENDSTAGE RENAL DISEASE

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It has been suggested that hypercoagulability associated with ESRD may be attributable to diminished circulating levels of antithrombin III (ATIII). Whole blood clotting and rheological assessment of blood, by thromboelastography (TEG) and Sonoclot (SCT) analyses, was performed on fresh specimens from 4 patient groups: control (C), hemodialysis (HD), peritoneal dialysis (PD) and well functioning kidney transplant recipients (Txp). TEG indices included r (rate of thromboplastin generation), r+k (coagulation time), Ang (rate of clot formation), MA (clot strength), LY30 and LY60 (% fibrinolysis at 30 and 60 minutes). SCT indices included SonACT (activation time) and SonR (rate of fibrin formation). ATIII levels were collected and assayed subsequently.

	C N=20	PD N=13	HD N=21	Txp N=20	P value
MA	64.5 ± 5.4	77.2 ± 4.2	73.4 ± 6.9	66.5 ± 13.8	<0.001 [A,B,D]
Ang	68.3 ± 8.8	74.1 ± 5.6	74.6 ± 4.7	64.1 ± 14.3	0.006 [C,D]
LY30	2.1 ± 1.8	0.7 ± 0.9	1.3 ± 1.3	1.5 ± 1.9	0.036
LY60	5.8 ± 3.8	2.5 ± 2.2	3.2 ± 2.7	3.4 ± 3.3	0.014 [A]
SonR	16.3 ± 4.3	32.4 ± 16.9	33.5 ± 29.3	24.5 ± 7.8	<0.001 [B]
SonACT	118.8 ± 19.2	145.4 ± 35.8	142.6 ± 41.9	127.6 ± 24.9	0.019
ATIII	84.6 ± 19.4	69.7 ± 20.6	68.7 ± 22.0	70.4 ± 22.3	0.049

P<0.05: A=PD vs C B=HD vs C C=HD vs Txp D=PD vs Txp

TEG and SCT measure quantitative and qualitative platelet and coagulation factor function. ESRD patients manifest thrombotic tendencies by TEG, SCT and ATIII levels. Txp appears to correct these values. Lowered levels of ATIII in both dialysis groups were not normalized post-transplant. It remains to be determined whether the benefits of transplant are attributed to platelet function or other coagulation factors.

TRANSPLANTATION AMELIORATES THE HYPERCOAGULABILITY OF ENDSTAGE RENAL DISEASE

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INTRODUCTION

Thrombotic complications in hemodialysis, peritoneal dialysis and kidney transplant patients

Thrombosis is a common complication of patients on hemodialysis and continuous ambulatory peritoneal dialysis (CAPD)(1) as clinically evident by the frequent rate of vascular access thrombosis which costs the United States $1 billion per year, and accounts for 14% to 17% of hospitalizations in this patient population(2). The incidence of thrombotic complications other than renal vein thrombosis in this patient population ranges from 8.5% to 44% with an overall incidence of 20%(3). The recent use of recombinant erythropoietin (rhEPO) has not generally seemed to change the frequency of thrombosis even though it has dramatically reduced the incidence of anemia among dialysed patients(4).

Etiologies for vascular access thromboses in patients on hemodialysis are divided into two categories: anatomic and non-anatomic causes. The overwhelming majority of anatomic defects consist of graft anastomosis stenosis, specially since the increase in utilization of synthetic grafts such as PTFE which are well-known to thrombose more commonly than native fistulas with a relative risk estimated in a recent surgical series of 1.98 over 1 year(5). Non-anatomic etiologies include a variety of systemic disorders, including inherited and acquired conditions. These conditions can be inherent to end stage renal disease (ESRD), such as hypertensive, diabetic or inflammatory

vasculopathies, or occur secondary to hemodialysis treatment, which can itself predispose to thrombosis via exposure to additional exogenous factors, loss of plasma proteins to dialysate, or poor anticoagulation management before, during, and after dialysis(6,7). Non-anatomic etiologies also include a broadly-defined, well-known condition referred by the literature as a "hypercoagulable state". This clinical condition, has remained a diagnosis of exclusion when thrombotic events cannot be accounted for by other known etiologies. The literature has presented many different laboratory criteria and diagnostic tests to characterize this condition, but it still remains loosely defined as "any alteration of the coagulation pathways that predisposes to thromboses"(8) largely because it is a highly complex, multi-factorial phenomena, whose underlying causes have not been well elucidated. Since platelet aggregation plays a major role in thrombus formation, antiplatelet agents such as Sunfinpyrazone, aspirin, and dipyridamole have been used with encouraging results(9).

Patients on CAPD, which is now an established form of therapy for ESRD, comprising 15% of the total number of patients worldwide on dialysis in 1997(10), have also been found to exhibit a hypercoagulable state(11). Prior studies have not characterized hypercoagulability in these patients since thrombosis is not a well-defined complication of CAPD. Unlike hemodialysis, which requires the use of heparin without a functioning kidney to metabolize it, peritoneal dialysis does not require the use of anticoagulant therapy and thus patients are less likely to develop bleeding complications. Nevertheless, characterizing hypercoagulability in this patient population could be very important since it remains controversial as to whether it places CAPD patients at an

increased risk for developing atherosclerosis and subsequent coronary artery and cerebrovascular disease.

Renal transplant recipients on immunosuppressive treatment are also well-known to exhibit a hypercoagulable state(12). Roughly 10-20% of kidney grafts are lost during the first year post-transplant(13). Acute rejection is the most common cause of rejection, but early graft loss due to venous or arterial thrombosis is a well known complication with an incidence of 1-7%(14,15). In pediatric patients, thrombosis is an even greater issue accounting for about 12% of failed transplant and 20% of failed repeat transplant(16). Recently, hypercoagulable states have also been implicated in certain forms of acute rejection (14). Biopsy specimens from patients with severe acute rejection frequently show intravascular fibrin deposition(17). This finding suggests that a hypercoagulable state could be a nonimmunologic factor playing an important role in acute rejection, and partly explains why despite the use of aggressive immuno suppressive agents, the incidence figures listed above have not decreased significantly over the past few years(18). Understanding, diagnosing, and managing hypercoagulability in kidney transplant patients could help prevent acute rejection as well as other long-term complications.

Thrombosis and the role of coagulation

Thrombosis is a multi-factorial, poly-regulated phenomenon that continues to be highly debated and controversial. Classically, Virchov's triad(19) has been used to

describe and compartmentalize the three main etiologic factors that lead to thrombosis: endothelial injury, low flow states, and hypercoagulability. As we advance our understanding of thrombosis, it has become apparent that these individual compartments are highly integrated and cannot be treated as individual clinical processes. Anatomic causes for thrombosis can be repaired surgically, underlying systemic conditions such as hyperestrogenic states and sickle cell anemia can be managed medically, but coagulopathic etiologies remain poorly elucidated, and poorly managed.

Normal hemostasis displays a delicate balance of ensuring that significant breaches in vessel walls are plugged, while at the same time preventing these same plugs from causing distal ischemia (Figure 1). This modulation consists of four general processes (modified from Robbins(8)):

1) Platelet activation:

   Following injury to a blood vessel wall, platelets are exposed to endothelial cell extracellular matrix (ECM) constituents such as collagen, proteoglycans, fibronectin and other adhesive glycoproteins. This exposure causes platelets to adhere to the vessel wall and to release of a variety of granules which lead to platelet aggregation and subsequent formation of a platelet plug, called *primary hemostasis.*

2) Coagulation cascade:

   Platelet granules also activate the coagulation cascade, which represents a series of conversions of inactive proenzymes to activated enzymes which culminates in the conversion of the plasma soluble fibrinogen into the insoluble fibrous protein fibrin.

Polymerized fibrin and platelet aggregates form a solid permanent plug that prevents further hemorrhage called *secondary hemostasis.*

3) Modulation of the coagulation cascade:

   Endothelial cells have the ability to prevent coagulation via a slew of membrane-associated inhibitory factors such as thrombomodulin, antithrombin III, protein C and protein S. This inhibitory mechanism, modulates the rate of prothrombotic activity and prevents unneccessary thrombosis.

4) Plasmin mediated fibrinolysis:

   Fibrin deposits are eventually cleared from endothelial surfaces through a process called fibrinolysis. This process is mediated via tissue type plasminogen activator (t-PA) synthesized by endothelial cells and urokinase-like plasminogen activator present in plasma and various tissues. Endothelial cells further modulate coagulation/anticoagulation by releasing plasminogen activator inhibitors that block fibrinolysis and confer an overall procoagulant effect.

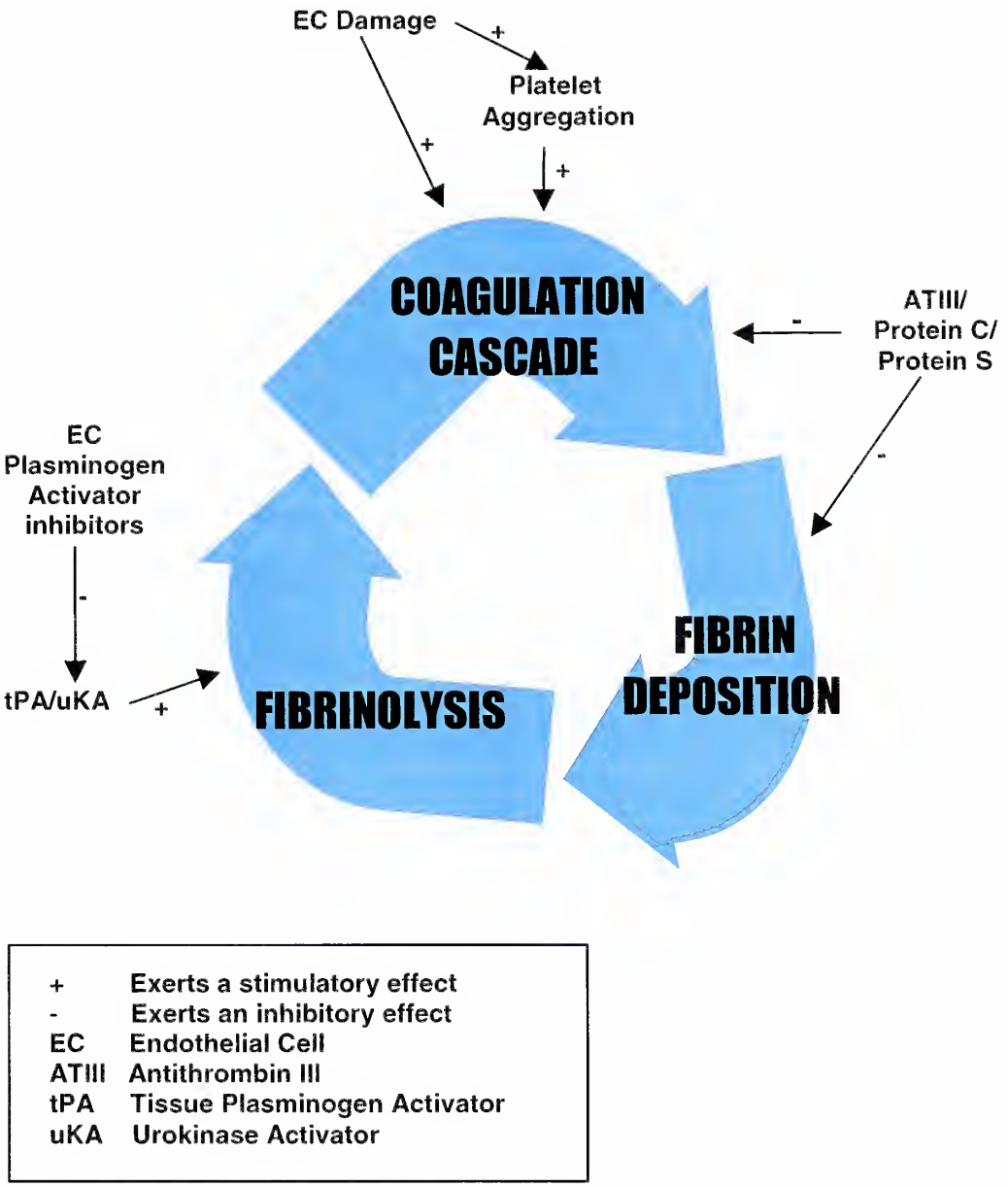


Figure 1 – Modulation of hemostasis in healthy individuals

Given the careful balance of forces modulating thrombotic activity, it is clear that abnormalities or imbalances in any of these processes could potentially tilt the balance towards a hyper or hypocoagulable state. There are several well characterized genetic conditions affecting specific hemostatic elements that lead to a hypercoagulable state such as factor V mutations, antithrombin III, and proteins C and S deficiencies. The most common of these is factor V Leiden mutation (FVL) consisting of a single point mutation in the factor V gene with an *Arg* to *Gln* transition at position 506 with a prevalence of 6% in Caucasian populations(20). It was observed that in patients with this point mutation, factor Va was resistant against the anticoagulant action of activated protein C leading to prothrombotic manifestations(21). In a surgical series, it was found that kidney allograft recipients with FVL had a 39% chance of developing venous thrombosis, including primary graft thrombosis, compared to a 15% chance in patients without the mutation(22). However, genetic risk factors alone do not explain hypercoagulability, and must be examined in the context of other prothrombotic conditions affecting hemodialysis, CAPD, and renal transplant patients which still remain ill-characterized.

Etiology of hypercoagulability in hemodialysis, peritoneal dialysis and kidney transplant patients

The literature contains a rich ensemble of studies (see below) exploring several different etiologies for hypercoagulability in hemodialysis, peritoneal dialysis and kidney transplant patients with limited agreement and elucidation. What follows is a comprehensive review of the findings that have helped shape our current understanding

of hypercoagulability in this population, with particular emphasis on the promising main players (Figure 2).

It is well known that the blood of patients on dialysis undergo protein loss via a mechanism resembling the nephrotic syndrome(23). Some papers argue that in addition to protein loss via dialysis, there is a significant alteration in the plasma protein content secondary to renal failure itself(24). Either way, levels of specific plasma elements get modified, break the careful hemostatic balance, and lead to coagulation abnormalities. A few studies have attempted to attribute hypercoagulability to abnormal levels of different coagulation cascade proteins in this patient population, and the following were described: hemodialysis patients had increased levels of factor VII(25), CAPD patients displayed increased activity of factors VII and VIII(26), and kidney transplant patients exhibited increased factor VII activity(27).

Other studies have attempted to explain hypercoagulability on the basis of antithrombotic protein deficiencies. Decreased levels of protein C have been found in CAPD patients(28) as well as increased levels of antithrombin III(26). In hemodialysis patients however, decreased levels of antithrombin III were found(28). These findings were supported in another series(29) which actually found that all three antithrombotic proteins (antithrombin III and proteins C and S) were more elevated in CAPD patients than in hemodialysis patients which was a somewhat equivocal finding since both dialysis modalities exhibit protein loss.

Abnormalities in fibrinolysis have also been described as a contributing factor to hypercoagulability in this patient population(30). Several series looking at t-PA activitity have found hyperfibrinolysis in both CAPD and hemodialysis patients (29,31). These studies have argued that the increase in fibrinolytic activity could be a response to a decrease in antithombotic substances, representing a backup antithrombotic protective mechanism. However, the finding of increased antithrombotic substances in CAPD patients(28,29) weakens this argument, which will remain unverified until fibrinolytic activity is directly correlated with actual antithrombotic substance levels. Kidney transplant patients on the other hand, have been shown to exhibit hypofibrinolysis(12). It remains unclear as to whether hypofibrinolysis is a potential primary process underlying hypercoagulability in kidney transplant patients.

In addition to abnormalities in the hemostatic processes, other plasma components have been associated with hypercoagulability in dialysis patients. Hyperhomocystenuria, a putative risk factor for atherothrombotic cardiovascular disease, was found to confer an increased risk for hemodialysis access thrombosis(32). Similarly, abnormal lipid metabolism and increased levels of atherogenic lipids, which is also a risk factor for coronary artery disease, was found to play a role in hypercoagulability and hyperfibrinolysis in CAPD patients (31). The latter finding was followed by a second study that looked at lipid reduction therapy as a way to correct hypercoagulability with unsuccessful results(33). The exact role of hyperhomocystenuria and abnormal lipid metabolism in promoting hypercoagulability in this patient population requires further examination.

More recent studies have noted that dialysis patients with high antiphospholipid antibody titers with and without systemic lupus erythematous were found to have more frequent access thrombosis than patients with low/negligible titers(34,35). Renal manifestations include thrombotic microangiography and large vessel thrombosis which is thought to be mediated via an autoimmune response. The mechanism underlying this phenomena has not been clearly delineated but it is thought to be caused by antiphospholipid antibodies directed against epitopes on oxidized phospholipids complexed with a glycoprotein, beta 2-glycoprotein I, or against the glycoprotein itself. Antiphospholipid antibodies may increase with time on dialysis, possibly as a result of oxidative stress incurred during dialysis(36). Moreover, higher than normal levels of antiphospholipid antibodies have also been found in kidney transplant patients(37). It is postulated that they are acquired pretransplant and persist in the body postransplant, and may help explain hypercoagulability in kidney transplant patients. Treatment of antiphospholipid antibody syndrome remains centered around anticoagulation with warfarin, since the use of immunosuppressive agents had no dramatic effect on titers and on the incidence of thrombotic events(36).

Hypercoagulability in kidney transplant patients is also thought to occur secondary to long-term steroid treatment(12,38). It is suggested that steroid use affects the fibrinolytic system, decreasing its activity and hence favoring a prothrombotic state. However, the largest prospective trial examining the effect of steroids on coagulation has failed to demonstrate a significant difference in thrombotic events between cyclosporine-

treated and non-cyclosporine-treated patients(14). Whether steroid use alone explains hypofibrinolysis in kidney transplant patients, or whether genetic as well as acquired risk factors predispose to this condition remains to be elucidated.

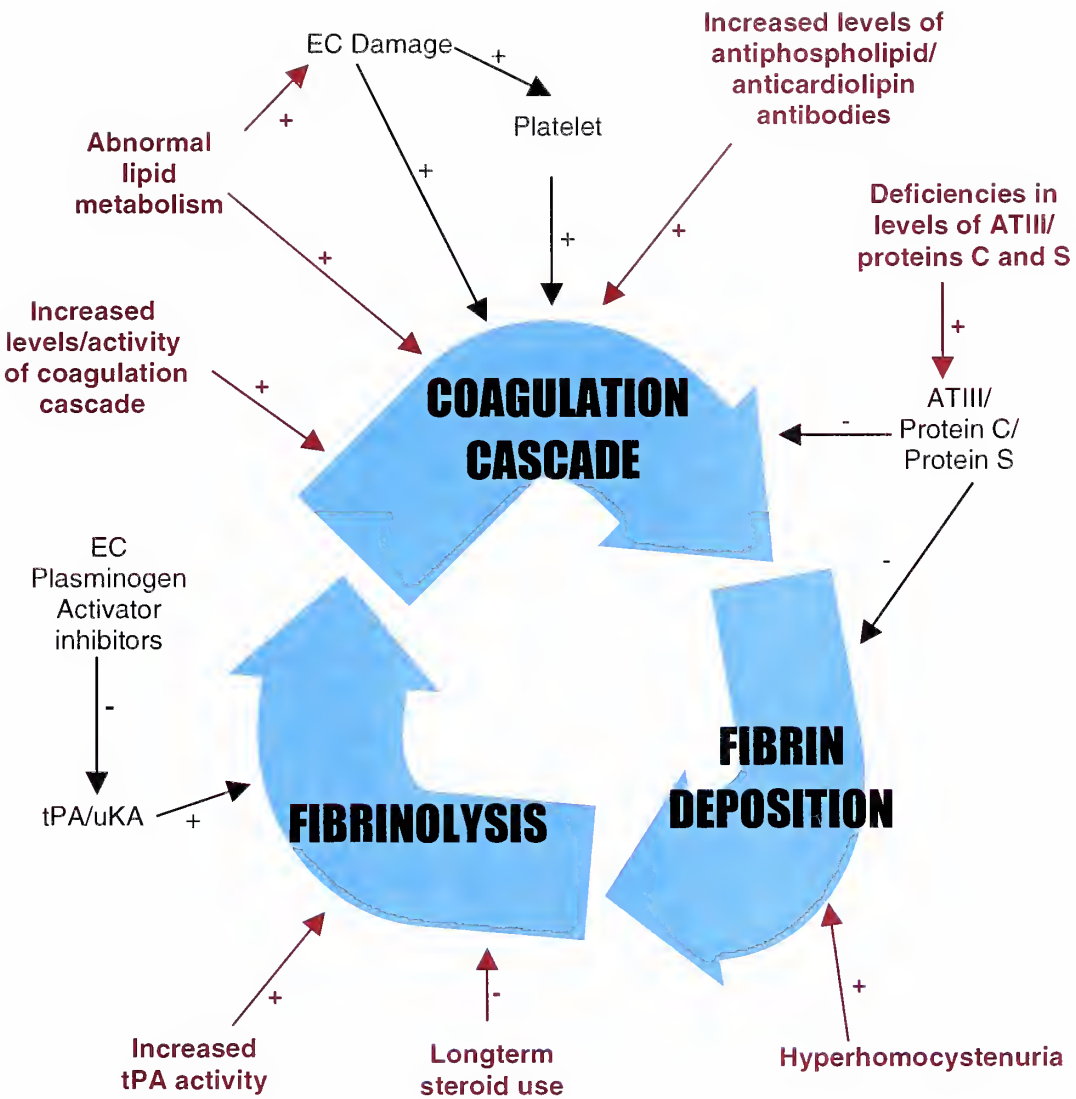


Figure 2 – Coagulation abnormalities

It has been shown that dialysis patients with elevated levels of anti-phospholipid antibodies experience a decline in their levels after a renal transplantation(37), suggesting that if indeed antiphospholipid antibodies are related to a hypercoagulable state, then kidney transplantation should improve the hypercoagulability in dialysis patients. However, Prior studies have not quantitatively compared hypercoagulability across hemodialysis, CAPD and kidney transplant patients to assess whether either modality of dialysis leads to a higher degree of hypercoagulability or whether kidney transplantation ameliorates hypercoagulability in dialysis patients.

Detection and management of hypercoagulability in renal failure: Is it economically viable to make it routine?

Dialysis and renal transplant patients are not routinely screened for hypercoagulability given the high costs associated with the tests and the limited evidence linking measurable parameters of hypercoagulabity and clinical thrombosis. On the other hand, costs associated with vascular access thrombosis and renal allograft thrombosis are also high. As mentioned before, these conditions costs the United States over $1 billion per year(39).

A recent study found that the incidence of renal allograft thrombosis could be reduced from 4% to 1.5% by anticoagulating renal transplant patients with laboratory evidence of hypercoagulability(40). The hypercoagulability panel employed in the study included ATIII deficiency, protein S deficiency, protein C deficiency, activated protein C

resistance and anticardiolipin antibodies. Patients with evidence of hypercoagulability were heparinized immediately post-op and maintained on Warfarin at a targeted INR of 2.0-2.5. Based on the successful reduction in the incidence of thrombosis, the authors subsequently performed a cost-benefit analysis to compare the costs of testing all renal transplant candidates for hypercoagulability with the savings incurred by reducing the incidence of allograft thrombosis. Their analysis determined that unless the hypercoagulability panel cost under $280, it was not cost-effective to test all candidates for hypercoagulability. The hypercoagulability panel employed in the study was estimated to cost $2200; an order of magnitude higher than necessary for cost-effectiveness.

Understanding the etiology of hypercoagulability would enable the development of more sensitive tools that could be used routinely to better manage renal failure patients. Increased sensitivity would theoretically enable a stronger reduction in the incidence of thrombosis which in turn, would allow more costly tests to achieve cost-effectiveness.

Thromboelastography and Sonoclot analysis as viscoelastic monitors of hypercoagulability in hemodialysis, peritoneal dialysis and kidney transplant patients

Studies looking at coagulation have for the most part analyzed individual assays of hemostatic components such as coagulation cascade factors, fibrinogen, antithrombotic substances, t-PA activity, fibrin degradation products among others. Lately, they have also included sophisticated assays of components such as proteins C and S activities, protein C resistance, factor II assays and antiphospholipid assays which include lupus anticoagulant, anticardiolipin antibodies and antiphosphatidyl serine antibodies(7). Coagulation studies also commoly describe hypercoagulability on the basis of abnormalities in prothrombin time (PT) and activated partial thromboplastin time (aPTT). Whereas these measurements are very effective screens for abnormal clotting, these tests only provide particular end-points in coagulation, and terminate when fibrin strands are formed. Each of these tests measure different components of coagulation, and even together, do not provide a comprehensive picture of the clotting process(41). In trying to understand coagulation abnormalities, which involves a complex array of interconnected factors, it would be more informative to obtain a comprehensive dynamic profile of a developing clot all the way from fibrin formation to clot dissolution.

Thromboelastography (TEG) and sonoclot analysis (SCT) are viscoelastic monitoring of blood clotting that provide a dynamic profile of coagulation. The basic principle involves the application of a mechanical force to coagulating blood, and

detecting the response(42). In other words, as coagulation begins, fibrin monomers linearly associate to form fibers and these subsequently cross link with each other and with red blood cells and platelet to form a clot. As this clot starts to develop, it becomes an obstacle for flow and its viscosity increases. If a mechanical force is applied to this developing clot, as viscosity increases there is increasing resistance to that force until it reaches a maximum when the clot is fully formed. As fibrinolysis commences, the clot begins to dissolute, the viscosity starts to decrease, and the resistance to flow drops accordingly. Thus, viscoelastic monitors such as TEG and SCT detect the liquid-to-solid transition of an evolving clot and provide a comprehensive profile of this transition rather than at end-points as in routine coagulation tests.

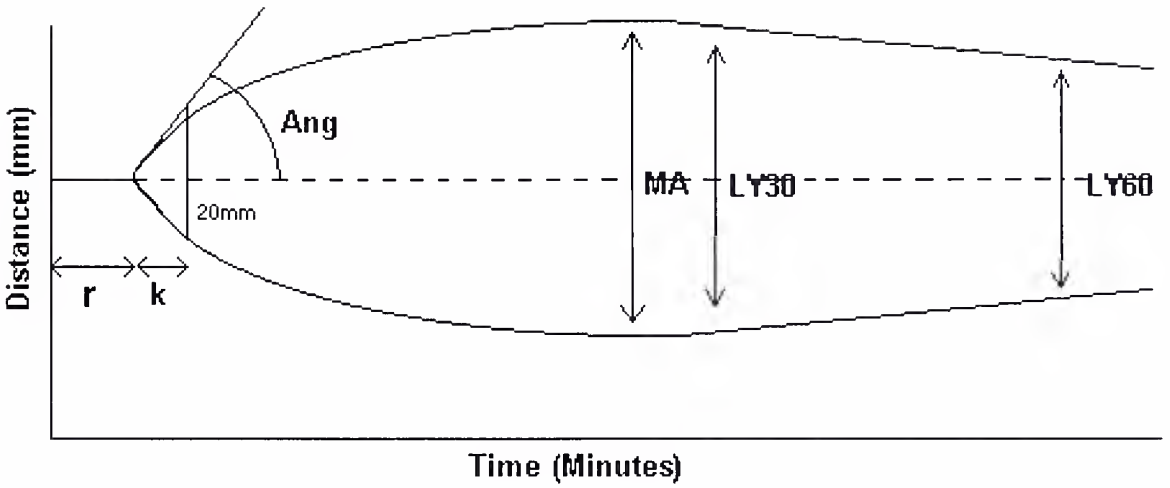
In TEG, 0.36ml of blood is placed into a TEG cuvette immediately following phlebotomy. A piston is lowered into the cuvette and as the cuvette is rotated back and forth around the piston, the increase in viscosity is transmitted through a torsion wire to a recording device that generates a curve over time. Figure 3 illustrates the TEG process and provides an explanation of the recorded indices. Figure 4 demonstrates the typical TEG pattern in the normal physiologic state and Figure 5 demonstrate the typical TEG in hypercoagulability. The TEG allows not only close monitoring of clot formation, but also gives great insight as to the activity of the fibrinolytic system(42).

In SCT, a hollow open-ended plastic probe mounted on a transducer is allowed to vibrate vertically while immersed in 0.4ml of fresh clotting blood. The viscous drag on the probe, impeding its free vibration, is detected by the electronic circuits driving the

probe and is converted to an output signal that is recorded on a paper chart. Figure 6 illustrates the SCT process and provides an explanation of the recorded indices. Figure 7 demonstrates the typical SCT pattern in the normal physiologic state and Figure 8 demonstrated the typical SCT signature in hypercoagulability (41).

The mechanical distinction between TEG and SCT is that TEG measures the torsional impedance imparted to a fixed piston suspended in a rotating cuvette of coagulating blood, whereas SCT measures the changes in impedance to movement of a vibrating probe in a developing clot (41).

TEG and SCT analysis have been shown to be superior over individual assays of hemologic components(43), particularly in detection and monitoring of the hypercoagulable state(44). The clinical usefulness of the TEG and Sonoclot analyzer is evidenced by its increased utilization for perioperative coagulation assessment in patients undergoing cardiopulmonary bypass and liver transplantation (41,45).



r **Rate of thromboplastin generation**

Time taken for the TEG amplitude to reach 2mm. It is a function of thromboplastin generation and the intrinsic coagulation system.

r+k **Coagulation time**

Time taken for the TEG amplitude to reach 20mm (i.e. clot of a certain strength). It is a function of the intrinsic coagulation system, platelets, and fibrinogen.

MA **Maximum amplitude**

Largest amplitude of the TEG. It is a function of clot strength. Improved quality of platelets, fibrinogen, and factor XIII increase MA.

Ang **Clot formation rate**

Rate of formation of a solid clot. It is a function of fibrinogen and platelet quality.

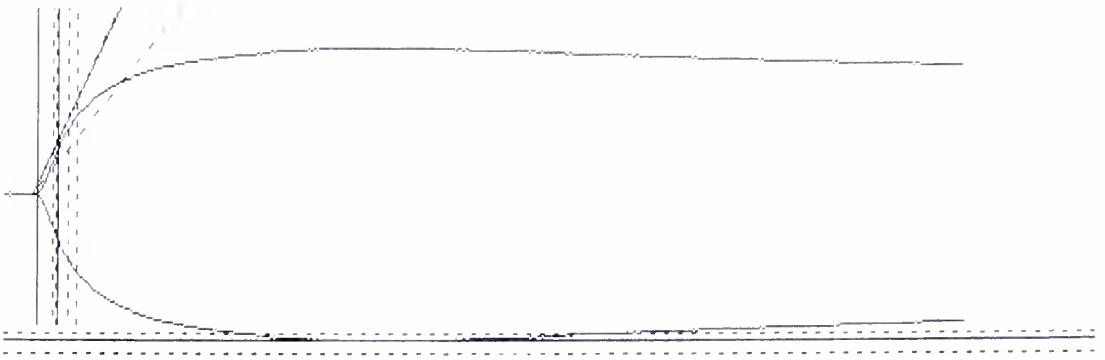
LY30 **Fibrinolysis at 30 minutes**

percentage decrease in TEG amplitude relative to MA at 30 minutes.

LY60 **Fibrinolysis at 60 minutes**

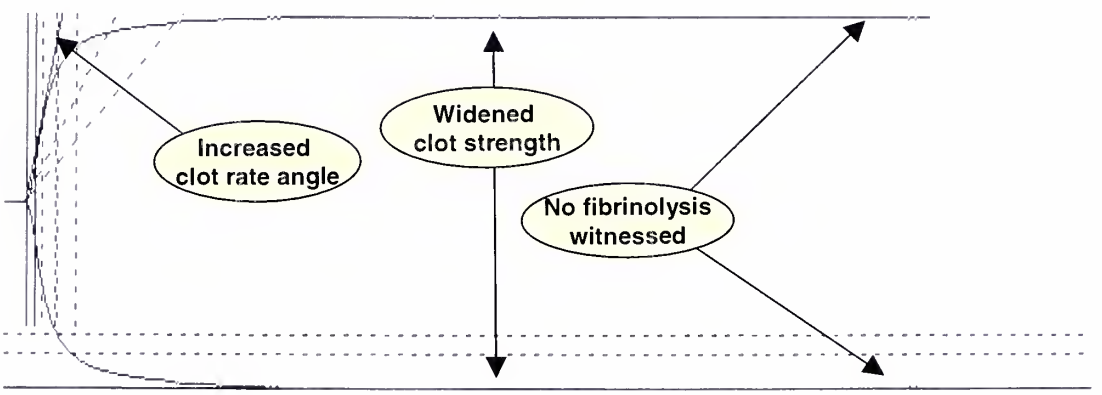
Percentage decrease in TEG amplitude relative to MA at 60 minutes.

Figure 3 – Schematic of indices recorded on TEG. Figure 4 and Figure 5 on proceeding pages show TEG of actual patients



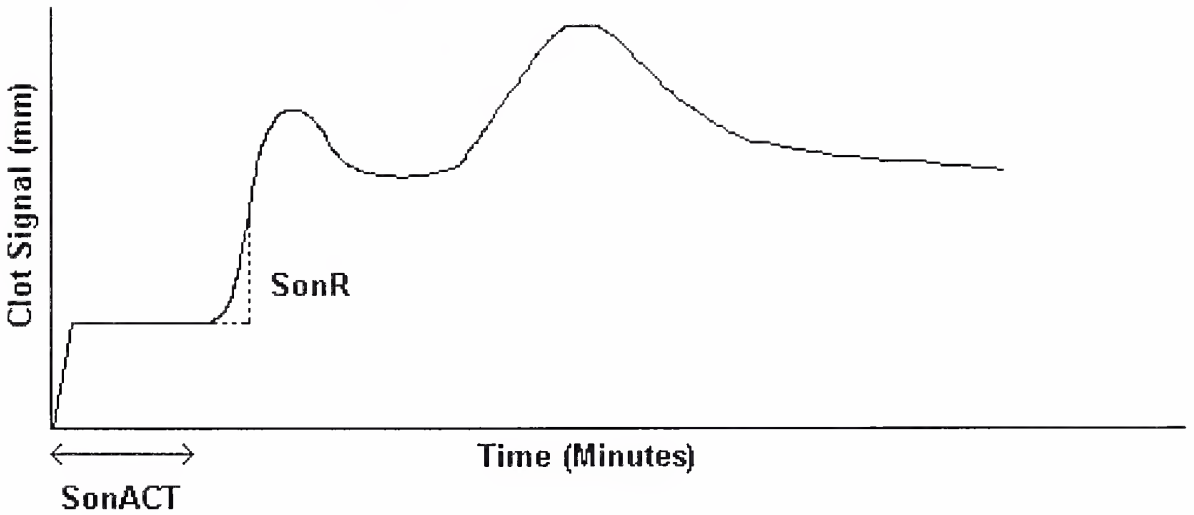
		R (mm)	K (mm)	MA (mm)	Ang (deg)	LY30 (%)	LY60 (%)
10 mm							
scale	Pt:	6.5	4.0	62.0	66.5	2.0	5.5

Figure 4 - TEG of a control patient



		R (mm)	K (mm)	MA (mm)	Ang (deg)	LY30 (%)	LY60 (%)
10 mm							
scale	Pt:	4.5	1.5	83.0	80.5	0.0	0.0

Figure 5 - TEG of a hypercoagulable patient. Hypercoagulability is demonstrated by an elevated clot strength (MA) and rate of clot formation (Ang) while impaired fibrinolysis is demonstrated by a lowered percent fibrinolysis at 30 and 60 minutes (LY30 and LY60).



SonACT **Activation time**

Time in seconds to the beginning of fibrin formation, defined as a 1mm upward deflection in the SCT signature.

SonR **Rate of fibrin formation**

Rate of solid clot formation which is directly proportional to the increase in viscosity.

Figure 6 – Schematics of indices recorded on SCT

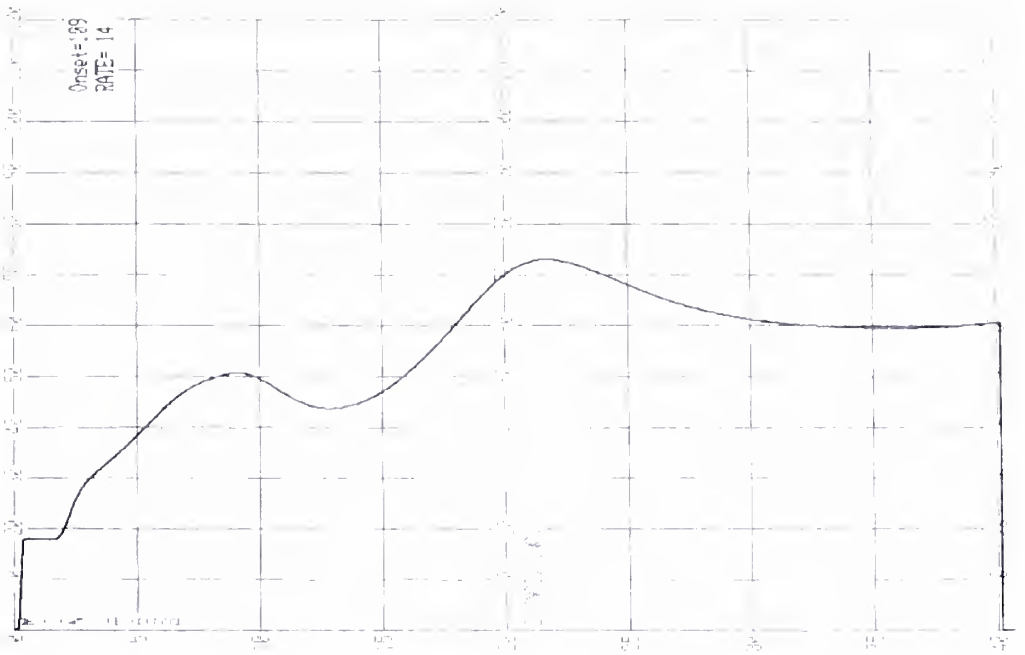


Figure 7 - SCT of a control patient

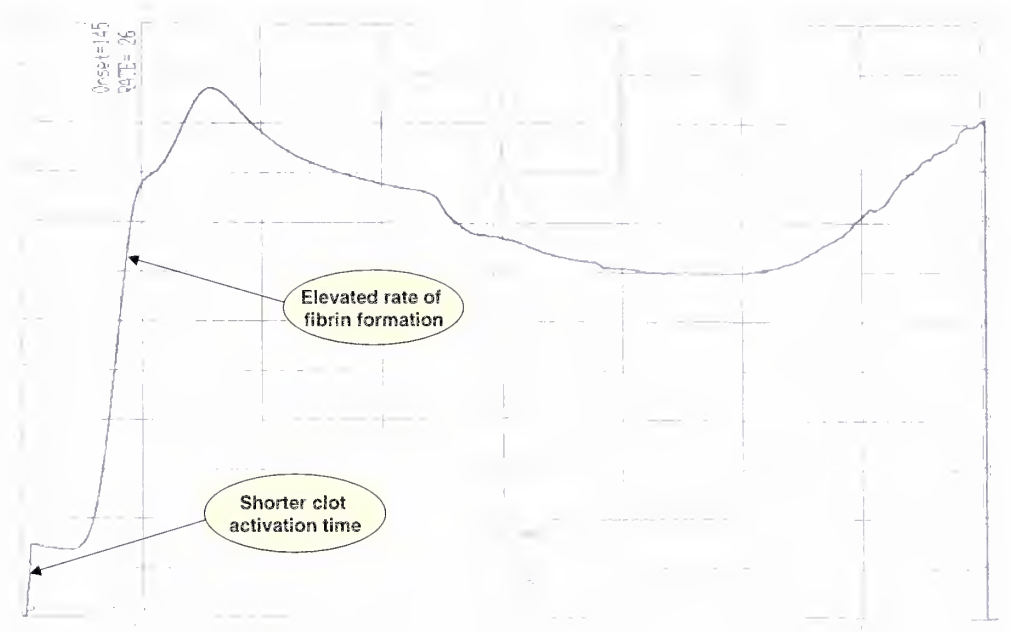


Figure 8 - SCT of a hypercoagulable patient. Hypercoagulability is demonstrated by a shorter clot activation time (SonACT) and by an elevated rate of fibrin formation (SonR).

STATEMENT OF PURPOSE

This study seeks to introduce thromboelastography (TEG) and sonoclot analysis (SCT) in the evaluation of hypercoagulability in hemodialysis, peritoneal dialysis, and kidney transplant patients. Most of the studies looking at coagulation in this patient population have used prothrombin time (PT), activated partial thromboplastin time (aPTT), protein levels, and assays of isolated haemostatic components to measure hypercoagulability. TEG and SCT were employed in this study since their measures of whole blood clotting and rheological properties in assessing hypercoagulability, have been demonstrated to be superior over assays of individual hematologic components(43,44).

The purposes of this study are threefold:

(1) to confirm a hypercoagulable state in hemodialysis, peritoneal dialysis and renal transplant patients compared to controls using thromboelastography (TEG) and sonoclot analysis (SCT);

(2) to compare hemodialysis and peritoneal dialysis patients;

(3) to determine whether the hypercoagulable state in dialysis patients is significantly corrected in kidney transplant patients; and

(4) to assay whether hypercoagulability in hemodialysis, peritoneal dialysis, and renal transplant patients is associated with low circulating levels of antithrombin III.

METHODS

Patient selection

From June 1998 to October 1998, 54 patients of Yale/New Haven Hospital volunteered to participate in this study and another 20 healthy volunteers participated as controls. The study participants were subsequently placed in one of four study groups accordingly: hemodialysis patients (N=21), CAPD patients (N=13), renal transplant patients (N=20) and healthy controls (N=20). There were 9 males and 12 females in the hemodialysis group with a mean age of 53 (range 11 – 74 years). There were 9 males and 4 females in the CAPD group with a mean age of 44 (range 35 – 61 years). There were 10 males and 10 females in the renal transplant group with a mean age of 49 (range 27 – 70 years) and 13 males and 7 females in the control group with a men age of 30 (range 19 - 58 years). Transplant patients had a mean cholesterol level of 241 (range 136-320), a mean triglyceride level of 207 (range 68-573), and had stable renal function (mean creatinine of 1.8 with a range 0.7-2.8).

All subjects were in stable condition. Exclusion criteria for all patients included any anticoagulant medication, and/or history of clinical hypercoagulability or active bleeding. Samples from hemodialysis patients were not obtained within 4 hours of a dialysis session. Phlebotomies in hemodialysis patients were done via peripheral veins.

All transplant patients were on different combinations of immunosuppressive therapies and were not within 3 months of a rejection period.

Blood sampling and data collection

Ten milliliters of blood were collected into vacutainer tubes for evaluation. PT, INR and PTT were measured using the HEMOCHRON, JR. Platelet count and Hematocrit were determined using a Coulter MD16.

Whole blood coagulation was determined in a computerized thromboelastograph™. Within 5 minutes of phlebotomy, 1 ml of whole blood was pipetted into a 1% celite suspension cup. The contents of the cup were then transferred to a $CaCl_2$ suspension. Following the transfer, 0.36 ml of the new mixture was pipetted into the thromboelastograph cuvette. The analyzer pin was then lowered into the cuvette. Mineral oil was placed on the surface of the sample to eliminate the blood-air interface and prevent drying during analysis. The coagulation process was recorded by computer. Recorded indices were R time (rate of thromboplastin generation), R+K time (coagulation time), alpha angle (rate of clot formation), maximum amplitude (clot strength) and % fibrinolysis at 30 and 60 minutes (refer to Figure 1 for explanation of TEG indices recorded. Refer to appendix for sample elastograph).

Whole blood coagulation was also determied using the SIENCO Sonoclot™ Coagulation and Platelet Function Analyzer. Within 5 minutes of phlebotomy, 1 ml of

whole blood was pipetted into a cuvette containing powdered celite. Immediately before analysis, a new plastic probe was inserted into the head and lowered into the cuvette. The coagulation process was recorded on thermo-sensitive paper. Recorded sonoclot indices included SonACT (fibrin formation time) and Scr (rate of fibrin formation) (refer to figure 3 for explanation of Sonoclot indices recorded. Refer to appendix for sample sonoclot recording).

5 ml of whole blood was centrifuged and the plasma was sent away for AT III levels.

Statistical analysis

Gender across the different study groups was compared with the Pearson chi-square test. Relationships between ATIII, platelet levels and hematocrit across all groups were assessed using Pearson Correlations. Relationships between TEG parameters and SCT parameters were also assessed using Pearson Correlations. General Linear Model (GLM) and multiple comparison contrast were used to assess continuous variables for multiple intergroup comparisons(control vs. PD, control vs. HD, control vs. TXP, TXP vs. HD and TXP vs PD). General Linear Model (GLM) and multiple comparison contrast were also used to assess continuous variables in men only for intergroup comparisons followed by female only analysis. P values reported on the GLM table are based on GLM and multiple comparison contrast except for gender which is based on chi-square. Values are reported as means and SDs.

RESULTS

Hematocrit:

Hematocrit values were significantly higher in the control group ($p<0.00001$) and in the transplant group ($p<0.01$) than in hemodialysis (HD) or peritoneal dialysis (PD) groups. Male hematocrit values were only significantly higher that females in the control group (males 42.4±3.1,n=13; females 37.4±3.8,n=7; $p<0.02$). In all other groups there were no significant differences.

Platelet levels:

There were no significant intergroup differences in platelet level. However, when correlated with TEG and SCT indices, platelet levels were strongly correlated with MA ($r=0.349$, $p=0.0022$) and with Ang ($r=0.333$, $p=0.0037$). There were no significant correlations between platelet level and Age, Hct, PT, INR, PTT or any other TEG or SCT indices.

Gender differences in coagulation:

For the most part, there were no significant gender differences in coagulation within the different groups with the exception of a few parameters that recorded significantly faster coagulation in females. In the control group, females had a significantly lower PTT (females 31.5±2.42; males 34.61±2.89; $p<0.03$), higher MA (females 68.1±3.0; males 62.6±5.4; $p<0.01$), and lower SCr (females 103±15.6; males 126±16; $p<0.02$). In the PD

group, females also had a significantly lower PTT (females 29.23±2.71,n=4; males 35.89±5.40,n=7; p<0.02).

Conventional coagulation tests:

PT and INR was significantly higher in HD patients than in controls (PT: control 14.0±1.2; HD 18.1±4.2; p<0.0009; INR: control 1.1±0.1; HD 1.4±0.3; p<0.001). There were no other intergroup differences in PT and INR. There were also no significant differences in PTT.

TEG:

HD and PD patients had a significantly higher MA (clot strength; p<0.001) and Ang (clot rate; p=0.006) by TEG than either control or Txp patients. K (coagulation time) was significantly higher in hemodialysis patients than in transplant patients (p=0.010), and LY60 (% lysis at 60 minutes) was significantly lower in PD patients than in controls. When controlling for Plt, differences remain significant (p=0.064 for K, p=0.001 for MA, p=0.0012 for ANG and p=0.01 for LY60). Differences also remain significant when data is controlled for Hct (p=0.02 for K, p=0.03 for MA and p=0.01 for ANG) with the exception of LY60 which loses its significance.

SCT:

HD patients had a significantly higher SCr (rate) than controls (p<0.001). No other groups demonstrated significant differences in SCr and after being controlled for HCT or

PLT the differences between HD and controls also lost their significance. Sco (onset) was not significantly different across the different study groups.

TEG correlated with SCT:

TEG parameters correlated with SCT parameters revealed the following significant relationships: SCT parameter SCr was strongly correlated with TEG parameters MA (r=0.4), Ang (r=0.324) and LY60 (r=-0.3) and SCT parameter Sco was strongly correlated with TEG parameters R+K (r=0.31) and MA (r=0.35).

ATIII:

There were no significant intergroup differences in AT III levels. However, ATIII levels were significantly correlated with Hct (r=0.35; p=0.0026), PT (r=-0.2; p=0.0346), and the TEG indices R (r=-0.3; p=0.03) and R+K (r=-0.2; p=0.0354).

RESULTS TABLES

Table 1: Characteristics of study groups

	Controls	Peritoneal dialysis	Hemodialysis	Transplant
N	20	13	21	20
Percent male	65.0%	69.2%	42.9%	50%
Age	30.0 ± 11	43.6 ± 8.4	52.8 ± 18.6	48.6 ± 14.4

Table 2: Comparison of conventional coagulation parameters across study groups

Parameters	Controls (C)	Peritoneal dialysis (PD)	Hemodialysis (HD)	Transplant (Txp)	P value
Hematocrit	40.6 ± 4.1	30.7 ± 4.6	31.2 ± 4.4	33.7 ± 6.0	< 0.001 a,b,c
Platelet count	225.1 ± 58.5	200.6 ± 77.4	182.5 ± 81.8	185.7 ± 94.3	0.317
PT	14.0 ± 1.2	16.5 ± 2.3	18.1 ± 4.2	16.0 ± 3.6	<0.001 b
INR	1.1 ± 0.1	1.3 ± 0.2	1.4 ± 0.3	1.2 ± 0.3	<0.001 b
PTT	33.5 ± 3.1	33.7 ± 5.6	34.3 ± 9.7	31.5 ± 3.7	0.413

p value comparison: a= C vs PD, b= C vs HD, c= C vs Txp, d= HD vs Txp, e= PD vs Txp

Table 3: Comparison of TEG parameters across study groups

TEG Parameters	Controls (C)	Peritoneal dialysis (PD)	Hemodialysis (HD)	Transplant (Txp)	P value
R	9.0 ± 4.2	9.9 ± 4.0	8.0 ± 2.5	11.5 ± 6.3	0.358
R+K	12.3 ± 5.7	12.5 ± 4.8	10.4 ± 2.8	15.3 ± 8.3	0.289
MA	64.5 ± 5.4	77.2 ± 4.2	73.4 ± 6.9	66.5 ± 13.8	<0.001 a,b,e
Ang	68.3 ± 8.8	74.1 ± 5.6	74.6 ± 4.7	64.1 ± 14.3	0.006 d,e
LY30	2.1 ± 1.8	0.7 ± 0.9	1.3 ± 1.3	1.5 ± 1.9	0.036
LY60	5.8 ± 3.8	2.5 ± 2.2	3.2 ± 2.7	3.4 ± 3.3	0.014 a

TEG Parameters: r= rate of thromboplastin generation; r+k= coagulation time; MA= maximum amplitude; Ang= clot formation rate; LY30 and LY60= fibrinolysis at 30 and 60 minutes (see Figure 3).

P value comparisons: a = C vs PD; b = C vs HD; c = C vs Txp; d = HD vs Txp; e = PD vs Txp

Table 4: Comparison of SCT parameters across study groups

SCT Parameters	Controls (C)	Peritoneal dialysis (PD)	Hemodialysis (HD)	Transplant (Txp)	P value
SonACT	118.8 ± 19.2	145.4 ± 35.8	142.6 ± 41.9	127.6 ± 24.9	0.019
SonR	16.3 ± 4.3	32.4 ± 16.9	33.5 ± 29.3	24.5 ± 7.8	<0.001 b

SCT Parameters: SonACT= activation time; SonR= Rate of fibrin formation (see Figure 6)

P value comparisons: a = C vs PD; b = C vs HD; c = C vs Txp; d = HD vs Txp; e = PD vs Txp

Table 5: Comparison of ATIII levels across study groups

	Controls (C)	Peritoneal dialysis (PD)	Hemodialysis (HD)	Transplant (Txp)	P value
ATIII levels	84.6 ± 19.4	69.7 ± 20.6	68.7 ± 22.0	70.4 ± 22.3	0.049

P value comparisons: a = C vs PD; b = C vs HD; c = C vs Txp; d = HD vs Txp; e = PD vs Txp

DISCUSSION

TEG and SCT in this study confirm the hypercoagulable state described in hemodialysis and peritoneal dialysis patients(1,23,24). Other studies have described this prothrombotic tendency using prothrombin time (PT), activated partial thromboplastin time (aPTT), protein levels, and assays and activities of isolated haemostatic components(25,26,27). We employed TEG and SCT in this study since their measures of whole blood clotting and rheological properties in assessing hypercoagulability have been demonstrated to be superior over assays of individual hemologic components (43,44).

TEG and SCT in this study also demonstrate that kidney transplant patients are significantly less hypercoagulable than patients in either dialysis groups, which suggests that kidney transplantation ameliorates the hypercoagulability found in ESRD patients on dialysis.

Several studies have attempted to characterize the underlying perpetrators of the hypercoagulable state without too much agreement nor elucidation. This study sheds new light on the following factors proposed by the literature to promote thrombosis:

AT III levels:

The literature displays contradictory findings as to the role of ATIII levels in hypercoagulability(3,46). In our study, ATIII levels were lower in HD and PD patients than in controls, which could help explain hypercoagulability in dialysis patients.

However, Txp patients were also found to exhibit equally low ATIII levels despite being less hypercoagulable than dialysis patients (according to other parameters recorded) which suggests that the coagulation amelioration found in transplant patients cannot be explained by ATIII levels alone. We conclude from these findings that while lower ATIII levels may weakly contribute to a hypercoagulable state, it is not the sole culprit.

Antiphospholipid antibodies:

Recent studies have suggested that antiphospholipid antibody levels may play a role in hypercoagulability in dialysis patients(34). It has been further shown that the levels of antiphospholipid antibodies present in dialysis patients decline after kidney transplantation(37). If antiphospholipids antibodies are indeed proven to be responsible for promoting a hypercoagulable state, then their decrease following kidney transplantation would help explain why coagulation after kidney transplantation is ameliorated as demonstrated by this study.

Anemia:

Hematocrit was lower in HD and PD patients than in both controls and kidney transplant patients which is an anticipated consequence of decreased erythropoietin secretion by the kidneys secondary to renal failure, and solute dilution by the dialisate. Anemia has been ruled out as a causative agent promoting hypercoagulability by studies that have demonstrated that while the increase usage of rhEPO amongst dialysis patients has significantly decreased anemia, it has not changed the incidence of thrombosis(4). Nevertheles, HCT in our study was strongly correlated with ATIII levels (r=0.35) which

suggests some biochemical connection between red blood cell and ATIII production. Whereas rhEPO may correct low HCT found in ESRD it may not correct levels of other coagulation factors that may be indirectly linked to red blood cell production.

Fibrinolysis:

Several studies looking at t-PA activitity have found hyperfibrinolysis in both CAPD and hemodialysis patients (29,31). Other studies have described a hypofibrinolytic state in kidney transplant patients(12). This study however, did not confirm these findings. It found no significant differences in fibrinolysis across the different study groups, with the exception of HD patients, who actually exhibited hypofibrinolysis when compared to controls. This contradiction may be partially explained by assay differences used to measure fibrinolysis: past studies measure t-PA activity and fibrin-split products, whereas TEG measures clot strength decreases over time as the clot within the TEG cuvette lyses, which provides a more direct measurement of fibrinolytic activity(42). Either way, it has been suggested that dialysis patients may experience an increased fibrinolytic activity as a response to decreased antithrombotic activity, which will remain unverified until fibrinolytic activity is directly correlated with actual levels/activity of antithrombotic substances in dialysis and kidney transplant patients.

Platelet count:

It is well known that in conditions where platelet levels are low or platelet activity is impaired, bleeding diatheses occur. Whereas no significant differences in platelet levels were found across different groups, there were strong correlations between platelet levels

and TEG indices in all groups, suggesting that platelet levels may contribute to hypercoagulability. However, even after the data was corrected for platelet count, HD and PD patients were hypercoagulable when compared to control patients suggesting that if platelet levels do indeed contribute to hypercoagulability, they are not the sole culprits. Relative platelet activity within the different study groups, which was not within the scope of this study, would be a useful measure to obtain in future studies investigating the role of platelets in hypercoagulability.

In addition to the factors included above, there are a several other substances/parameters that have been studied in order to explain hypercoagulability in dialysis patients. These studies have not found a strong association between any of these components and a hypercoagulable state which can only mean one of three things:

1. No study has effectively demonstrated which single hemostatic component is the best predictor of coagulation complications
2. Despite the variety of plasma components currently being investigated for their link to hypercoagulability, there could be other components that have not yet been identified.
3. Given the fact that no single component is a good predictor of coagulation complications, it is very likely that the underlying cause of hypercoagulability in this patient population may be a combination of possible culprits acting in unison.

This study, for example, was not able to strongly correlate ATIII levels to TEG or SCT indices, but the possibility still remains that ATIII levels may underlie hypercoagulability

in some of the patients in the study whereas other patients demonstrate hypercoagulability secondary to other factors such as antiphospholipid antibodies. If that were the case, this study would have had to look at correlations between TEG/SCT indices and low ATIII/high antiphospholipid antibodies at the same time. In addition, there are genetic risk factors that can predispose any one of the conditions above and lead to variability in these studies. Only by collecting data on all potential plasma components identified by the literature as possibly playing a role in hypercoagulability, and performing multivariate analysis to compare them with TEG and SCT profiles would such complex multi-factorial correlations be found. Medicine classically attempts to find one answer that explains several phenomena, but given the complexity of coagulation and the years of unsuccessful pursuit for a single culprit to explain hypercoagulability, it might be time to pursue multi-factorial answers. Due to the increase in shortage of organs, screening of genetic and acquired risk factors for hypercoagulability needs to be considered to stratify risk and allow rational intervention.

Since TEG and SCT provides a comprehensive picture of coagulation regardless of underlying culprits, they could be used to predict the risk of renal allograft rejection and thrombosis in transplant candidates. If a correlation is demonstrated between TEG and SCT tracings and the risk of rejection and thrombosis then transplant candidates with hypercoagulable tracings could be prophilactically anticoagulated post-op.

The seemingly more rapid coagulation in females as evidenced by PTT, MA and SCr in the control group and PTT in PD group is consistent with findings by Francis et

al.(47) who used TEG and SCT to investigate hypercoagulability in cancer patients. This gender difference cannot be explained by the scope of this experiment and requires further investigation.

Finally a few words about TEG and SCT. Several parameters of TEG and SCT were well correlated with each other in this study which confirms the validity of these tests as accurate monitors of coagulation. Whereas we recommend the use of TEG and SCT in future studies investigating coagulation abnormalities, we also have a few words of caution. While TEG and SCT manuals recommend analysis within 5 minutes of phlebotomy, parameters that monitor the onset of clotting(R and R+K in TEG and SonACT in SCT) will differ significantly if blood samples are analyzed 5 minutes as opposed to 30 seconds after sampling. The same holds true for measures of PT, INR and PTT using the HEMOCHRON, JR analyzer. It was no surprise that these parameters were not reliably statistically significant after data analysis. We therefore recommend that investigators attempt to analyze blood at a constant set point, say 2 minutes after phlebotomy, so that variation is minimized and that the machine be placed in a centralized location, for easy access from all sample collection stations.

In summary, this study utilized TEG and SCT to confirm that a hypercoagulable state is present in hemodialysis and peritoneal dialysis patients, and to demonstrate that kidney transplantation ameliorates the hypercoagulability found in dialysis patients. ATIII levels could not account for the amelioration observed in this study and other possible etiologies could not be verified by the scope of our study. Future studies

attempting to identify with confidence the underlying causes of hypercoagulability need to assay levels and activity of all potential culprits suggested by the literature, and correlate them in combination to all TEG and SCT parameters.

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